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Delaval, Jan

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Art unit 1644
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sites appear to be utilized in the murine and human cell lines. Several studies have shown that cell specific N-glycosylation can modulate the HA binding function of CD44. Cell lines and normal B-cells showed differences in N-glycosylation associated with different HA binding states. In particular, CD44 from HA binding cells had less glycosylation than from non-HA binding cells. Additionally, removal of sialic acids (both from the cell surface and from CD44-Ig fusion proteins) enhances HA binding. Thus the HCELL CD44 glycoform of the invention is unlike any previously described CD44.

In contrast the non-conserved region (~aa 183 to 256) shows only ~35% similarity between mammalian species. This region contains potential sites for numerous carbohydrate modifications of CD44 and the site of alternative splicing which allows for the insertion of extra amino acid sequence from variable exons of the CD44 gene.

A HCELL polypeptide comprises an amino acid sequence of CD44 and binds to antibody having the binding specificity of monoclonal antibody HECA-452. (ATCC Number: HB-11485) HECA-452 recognizes cutaneous lymphocyte associated antigen. HECA-452 binding of HCELL decreases after N-glycosidase-F, sialidase or fucosidase treatment. Furthermore, HCELL activity, *e.g.*, E-selectin and L-selectin binding also decreases upon N-glycosidase-F, sialidase, or fucosidase treatment demonstrating the importance of the sialofucosylated N-linked glycans in HCELL function. In contrast, sialylation of CD44 inhibits binding of CD44 to hyaluronic acid. Moreover, CD44 binding to hyaluronate is increased by sulfation, but sulfation is not necessary for the E- and L-selectin activity of HCELL.

Preferably the CD44 polypeptide is the standard or hematopoietic isoform of CD44 (CD44H). Alternately, the CD44 polypeptide is the R1 (CD44R1) or R2 isoform (CD44R2). For example, a HCELL polypeptide comprises the amino acid sequence of SEQ ID NO:1. (Gen Bank Acc. CAA40133; Table 1) A HCELL polypeptide is at least about 30%, 50%, 70%, 80%, or 95% identical to the polypeptide sequence of SEQ ID NO:1.

Table 1

1	mdkfwwhaaw	glclvpplsla	qidlnitcrf	agvfhvknkng	rysisrteaa	dlckafnstl
61	ptmaqmekal	sigfetcryg	fiaghvvipt	ihpnsicaan	ntgvyiltsn	tsqdytycfn
121	asappeedct	svtdlpnaf	gpititivnr	dgtryvqkge	yrtnpediyp	snptdddvs
181	gsserssts	ggyifytfst	vhpipedesp	witdstdrip	atnmdsshst	tlqptanpnt
241	glvedldrtg	plsmttqgsn	sqsfstsheg	leedkdhptt	stltssnrnd	vtggrrdpnh
301	segsttlleg	ytshyphkte	srtfipvtsa	ktgsfgvtav	tvgdnsnvn	rslegdqdtf
361	hpsggshth	gsesdghshg	sqeggantts	gpirtqppe	wliilaslla	lalilavcia
421	vnsrrrcgqk	kklvinsgng	avedrkpsgl	ngeasksqem	vhlvnkesse	tpdqfntade

HCELL POLYPEPTIDES

One aspect of the invention pertains to isolated HCELL glycoproteins, and biologically active portions thereof, or derivatives, fragments, analogs or homologs thereof. Also provided are polypeptide fragments suitable for use as immunogens to raise anti-HCELL antibodies. In one embodiment, native HCELL glycoproteins can be isolated from cells or tissue sources by an appropriate purification scheme using standard protein purification techniques.

In another embodiment, HCELL glycoproteins are produced by recombinant DNA techniques. Alternative to recombinant expression, a HCELL glycoprotein or polypeptide can be synthesized chemically using standard peptide synthesis techniques.

An "isolated" or "purified" protein or biologically active portion thereof is substantially free of cellular material or other contaminating proteins from the cell or tissue source from which the HCELL glycoprotein is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of HCELL glycoprotein in which the protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of HCELL glycoprotein having less than about 30% (by dry weight) of non-HCELL glycoprotein (also referred to herein as a "contaminating protein"), more preferably less than about 20% of non-HCELL glycoprotein, still more preferably less than about 10% of non-HCELL glycoprotein, and most preferably less than about 5% non-HCELL glycoprotein. When the HCELL glycoprotein or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the protein preparation.

The language "substantially free of chemical precursors or other chemicals" includes preparations of HCELL glycoprotein in which the protein is separated from chemical precursors or other chemicals that are involved in the synthesis of the protein. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of HCELL glycoprotein having less than about 30% (by dry weight) of chemical precursors or